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ALTERNARIA ROT OF LEMONS

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INTRODUCTION

With the possible exception of Penicillium, Alternaria is probably instrumental in causing more California lemons to decay than any other known fungus. That the same may be true for other lemon-producing localities is indicated by the following quotation: "In six cars of lemons from Italy, from 4 to 55% or an average of 18% of Alternaria-infected lemons was found."[3] The same article states that while this survey was being made ninety-one cars of lemons from California showed an average of 10 per cent of Alternaria rot.

In this connection it is of interest that Fawcett[4] found, as a result of a study of the lemons shipped to the central and eastern markets of the United States during the latter part of July and in August, 1924, that under the conditions prevailing at that time Alternaria was responsible for more decay than all of the other fungi combined.

Alternaria rot develops in lemon fruits almost exclusively under storage, transit or market conditions. It appears in the unpicked fruit only when it is allowed to become over-ripe or when some abnormal condition, such as a freeze, has materially weakened the fruit. As will be shown later on in this paper, hundreds of tests have proved that practically every fruit is potentially infected by the time it is a few weeks old; but the development of the fungus and the destruction of the fruit is delayed by the vitality of the fruit.

During the early part of this study decaying fruits were obtained from a large number of the lemon packing houses in southern California and a few from the central and northern parts of the state. These fruits were supposed to be infected with Alternaria and cultural tests confirmed the supposition. The results of these tests led to the conclusion that the fungus is prevalent in all lemon-growing districts of the state. While subsequent observations have confirmed this, it

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[3] Anderson, H. W. Plant Disease Survey Supplement **14**: 111. 1921. (Reported by the Inspector for the U. S. D. A. Bureau of Markets.)

[4] Fawcett, H. S. The decay of citrus fruits on arrival and in storage in eastern markets. Calif. Citrograph **10**: 79, 98–99, 103. 1925.

was observed also that the fruit from certain districts or from individual packing houses showed more decay than that from other sources. This is probably due to variations in the vitality of the fruit or else to differences in the packing house conditions. It cannot be too strongly emphasized that any storage, transit or market condition that tends to weaken the fruit will also tend to promote the development of Alternaria, since this fungus usually develops very slowly, if at all, in lemons of high vitality.

A systematic study of this species of Alternaria has not been made. However, its behavior in artificial cultures indicates that at least two forms or strains, if not species, may produce the decay in question. In some respects the resemblance to *Alternari citri,* which causes black rot of the navel orange, is striking, but whether the same organism is involved in the two cases has not yet been determined.

In the eastern markets especially, one type of this diseased condition of the lemon is referred to as ''center rot,'' because of its characteristic invasion of the internal tissues before it is evident on the surface of the fruit. However, there are other fungi which cause a similar decay of the lemon and for this reason it seems best to use here the term ''Alternaria rot'' rather than the less specific term, ''center rot.''

ALTERNARIA ROT AND ENDOXEROSIS COMPARED

The terms Alternaria rot and endoxerosis[5] should not be used interchangeably. Until 1920 the decay in lemon fruits, now known as Alternaria rot, was considered by the growers and packers to be the advanced stages of endoxerosis. At that time an intensive study of the disease was begun. Boxes of yellow, silver, and green lemons, half of each lot being sound and the other half affected with endoxerosis, were carefully selected and placed under the usual storage conditions in each of five packing houses. Once every month for four months a portion of the fruit in each lot was cut to detect any increase in percentage of lemons showing endoxerosis and any evidence of its increase in severity in those lemons affected when placed in storage. Any of these lemons which showed a more marked breaking down of the tissues, either externally or internally, as well as samples of those which had remained the same, were brought to the laboratory where cultures were made from the tissues.

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[5] Endoxerosis (pronounced ''en-do-zer-ó-sis,'' means internal drying) is a technical term used in place of the less definite terms locally applied to this malady, such as ''internal decline,'' ''blossom-end decay,'' ''dry tip,'' and ''yellow tip.''

The observations in the packing houses and the results of the cultural tests appeared to justify the following conclusions: (*a*) the healthy fruits did not become affected with endoxerosis, (*b*) those already affected with endoxerosis did not show any increase of the characteristic symptoms, (*c*) in the cultures no fungus could be isolated from the healthy fruits or from those having only the symptoms of endoxerosis as it appears in the fruits taken directly from the trees, and (*d*) the cultures showed that actual decay of the healthy lemons and of those having endoxerosis was caused in every case by a fungus. In almost every case Alternaria was found to be the cause of the decay except, of course, where molds had gained entrance through mechanical injuries due to handling. In only a few isolated cases were such fungi as Colletotrichum and Phomopsis found to be the principal causes of the decay.

In 1921 a similar experiment was conducted in collaboration with 15 lemon association packing houses. The houses were widely distributed over the lemon-growing districts of southern California. After the experiment was started the individual tests were put into the care of the manager or foreman, or both, of the respective packing houses with the instruction (*a*) that they make the observations, (*b*) that they send fruits to the laboratory for making cultures, and (*c*) that upon the termination of the test they report their conclusions.

The results of the cultural tests were identical with those made in 1920. The breaking down of the tissues of those fruits affected with endoxerosis did not progress, unless they were infected by Alternaria. Reports from thirteen of the packing house managers or foremen showed that no sound lemons became affected with endoxerosis after they were placed in storage. Of the other two, one reported inconclusive results and one reported that "The decline (endoxerosis) continued to advance in the affected fruit resulting in a final complete drying-up or slushy rot." This last statement does not in any way contradict the conclusion that endoxerosis does not progress in the fruit after it is taken from the tree because the symptoms mentioned are those of Alternaria and not of endoxerosis. The statement serves to indicate the confusion previously existing as to the identity of the two diseases. To prevent future confusion it may be well at this time to state the characteristic symptoms of these two maladies. Plate 1 illustrates many of the characteristic differences between these two diseases.[6]

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[6] See also Fawcett, H. S., and H. A., Lee. Citrus diseases. McGraw-Hill Book Co., N. Y. (For Alternaria rot see fig. 130, A to D, for endoxerosis see fig. 149.)

	Alternaria rot	*Endoxerosis*
1. *Cause*	Fungus (Alternaria).	No fungus (physiological).
2. *Origin in fruits*	Stem end, just under button,* peel usually last to be infected.	Stylar end, most pronounced in peel.
3. *External color of affected fruit*	Light to very dark brown.	Light lemon-yellow to orange; in green fruit often no abnormal color in early stages.
4. *Color of internal tissues*	Pink to light brown in very early stages; brown to almost black in late stages.	Pinkish to straw-yellow in early stages; light straw-brown in late stages.
5. *Gum formation*	Usually none, in some cases a trace in the core just under button.	Much in core at stylar end of fruit and in splotches in the peel, especially in early stages.
6. *Tissues attacked*	All tissues of both peel and pulp, in advanced stages.	Visibly only local portions of interior of peel and one-quarter to one-half of pulp in outer end of fruit in advanced stages.
7. *Disintegration of tissues*	Much in both peel and pulp; almost complete in late stages.	Comparatively little in either peel or pulp; mostly collapse, due to drying.
8. *Other effects on tissues*	Tissues become soft and watery; fruit loses its original shape in late stages.	Tissues of both peel and pulp remain comparatively firm, except as affected by the withdrawal of water; fruit retains original shape, except that in severe cases a slight depression may form on one side or all around at the base of the ''nipple.''

* The button is that portion of the stem which is left attached to the lemon fruit when it is picked; it consists chiefly of calyx and receptacle.

The results of these two sets of experiments, performed in 1920 and in 1921, together with widespread observations since that time, have shown conclusively that the breakdown and decay of the fruits in question is due to the action of the fungus Alternaria and not to the so-called "later stages" of endoxerosis.

INOCULATION EXPERIMENTS

During the course of the preceding observations and experiments the question arose as to whether the lemon fruits became infected with Alternaria before or after being picked. An effort was made to answer this question by making inoculation experiments first in two packing houses and later under controlled conditions in the laboratory.

TABLE 1

INOCULATION EXPERIMENTS IN THE PACKING HOUSE. COMPARATIVE PERCENTAGES OF ALTERNARIA ROT APPEARING IN INOCULATED AND UNINOCULATED SOUND AND ENDOXEROTIC LEMONS AFTER 90 DAYS IN STORAGE.

Condition of lemons	Proportion of lemons affected with endoxerosis	Lemons infected by Alternaria		Average percentage of Alternaria
		Inoculated	Checks	
	Per cent	*Per cent*	*Per cent*	*Per cent*
Yellow, sound	20	17	6	12
Yellow, endoxerotic	75	35	25	30
Silver, sound	15	9	9	9
Silver, endoxerotic	50	20	30	25
Green, sound	10	5	3	4
Green, endoxerotic	15	10	6	8

In the packing houses.—Two hundred lemons of each of the kinds indicated in table 1 were taken from the washer and dried. Before these fruits were placed under the usual storage conditions, those designated as "inoculated" were "painted" with a suspension of Alternaria spores, usually on the stem end around the button but in some cases on both stem and stylar ends. At the end of each of three 30-day intervals the percentages of Alternaria appearing in each lot of fruit were determined (table 1). The fruit in one packing house showed a slightly higher percentage of Alternaria in each lot than that in the other house, but the ratios between the different kinds of fruit were similar.

The results are not conclusive. They show a higher percentage of Alternaria rot in the inoculated yellows and greens but not in the silvers. While the results show that in four of the six lots the amount

of Alternaria rot appearing within this three-month period was apparently increased through inoculation, yet they clearly do not prove that none of the lemons in any of the lots would have decayed without inoculation. The results of some of the experiments to be described a little farther on in this paper will give additional evidence on this question. No infections resulted from the stylar-end inoculations.

A point of particular interest in connection with this experiment is the fact that a much larger percentage of Alternaria rot appeared in the endoxerotic than in the sound lemons, as indicated in the last column of table 1. These and other experiments and observations indicate that this was due to the lower vitality of endoxerotic lemons, which makes them less able to resist invasion by the fungus.

In the laboratory.—The inoculation experiments in the laboratory were confined to silver and light-green lemons. They were carefully sorted and only those of the best quality were used. Each group of silvers and light greens was divided into twenty-one lots of twenty lemons each. Of the twenty-one lots of silvers seven were used as controls, seven were soaked five minutes in copper sulphate solution ($1/50$ of 1 per cent, as commonly used in the lemon washer in the packing houses) and the other seven, after soaking as in the preceding case, were dried and then inoculated with a suspension of a pure culture of Alternaria spores in water. The suspension was applied by means of a camel's-hair brush and, in so far as possible, was applied under as well as around and on top of the buttons. The twenty-one lots of light greens were divided and treated in the same manner. All lemons were wrapped in standard wrappers. The lots of silvers and light greens were then divided into seven groups in such a manner that each group contained one lot of silvers for controls, one that had been soaked, one that had been soaked and then inoculated and three similar lots of the light greens. Each group therefore included sixty silvers and sixty light greens. One of these seven groups was then placed in each of the seven compartments of a constant temperature apparatus. The respective temperatures of these compartments were approximately 48°, 59°, 68°, 75°, 82°, 90° and 97° F. The temperature in any one compartment did not vary more than one or two degrees during the course of the experiment. The relative humidity in each compartment was at or near the saturation point. At intervals of from one to three weeks for a period of three and a half months the fruit was examined and the number in each lot that showed visible signs of decay was recorded and cultures made, when necessary, to identify the fungus causing the decay. A summary of the results of this experiment is shown in table 2.

TABLE 2

EFFECT OF ATTEMPTED STERILIZATION AND OF ARTIFICIAL INOCULATION ON THE AMOUNT OF ALTERNARIA ROT APPEARING IN SOUND SILVER AND LIGHT-GREEN LEMONS, UNDER CONTROLLED TEMPERATURE CONDITIONS.

Kind of lemons	Treatment	Per cent Alternaria rot at the following temperatures, °F							Average percentage Alternaria rot
		48	59	68	75	82	90	97	
Silvers	Checks	0	0	5	10	40	50	20	18
	Soaked in $CuSO_4$	10	10	25	20	40	35	15	22
	Soaked in $CuSO_4$, inoculated	15	20	15	30	40	50	20	27
Greens	Checks	0	5	0	10	45	25	25	16
	Soaked in $CuSO_4$	0	0	5	15	35	20	25	14
	Soaked in $CuSO_4$, inoculated	0	5	0	5	55	35	35	19

The results of this experiment show that:

1. The copper sulphate solution had no sterilizing effect on the lemons, in so far as preventing development of Alternaria rot was concerned.

2. Artificial inoculation of the lemons apparently increased to a limited extent the percentage of Alternaria rot appearing in the lemons, within the time limits of the experiment, as was also shown in table 1. However, the data in table 3 should be taken into consideration in this connection. It may be said also that the water added at the time of making the inoculation was probably an important factor in increasing the amount of decay in the inoculated lemons; the added water increased the amount of moisture under the buttons and thus made conditions more favorable for the growth of the Alternaria.

3. The optimum temperature for the development of Alternaria in these lemons proved to be in the neighborhood of 82° F. Subsequent experiments and observations have shown that this is approximately the optimum temperature for the development of Alternaria rot in lemons in general.

On the date of the last examination all of the lemons were removed from the constant-temperature apparatus. A large number of them still showed no exterior indications of decay. This was especially true of those that had been kept at the lower temperatures. As the lemons were removed from the compartments each was cut transversely just below the button to detect any case of Alternaria rot in the early

stages of development. The percentage in each compartment showing the development of Alternaria is given in table 3. The extent of invasion of the Alternaria, from the buttons into the central tissues of the lemons, ranged from about an eighth to three-quarters of an inch.

TABLE 3

PERCENTAGES OF THE EXTERNALLY SOUND LEMONS OF TABLE 2, WHICH, WHEN REMOVED FROM THE COMPARTMENTS OF THE CONSTANT-TEMPERATURE APPARATUS AND CUT ACROSS JUST BELOW THE BUTTONS, SHOWED THAT ALTERNARIA HAD BEGUN TO INVADE THE TISSUES.

Temperature °F	Per cent silvers having Alternaria rot			Average	Per cent greens having Alternaria rot			Average
	Controls	Soaked in $CuSO_4$	Soaked in $CuSO_4$, inoculated		Controls	Soaked in $CuSO_4$	Soaked in $CuSO_4$, inoculated	
97	100	100	100	100	100	100	100	100
90	100	100	100	100	98	96	99	98
82	100	98	99	99	91	89	88	89
75	100	91	92	94	78	75	78	77
68	79	69−	76	75	70	79	60	70
59	55	72	69	65	42	45	42	43
48	55	61	59	58	35	45	40	40
Average....	84	84	85	84	73	76	72	74

The data in table 3 show:

1. A large percentage of the apparently sound lemons was found to be already infected with Alternaria in the early stages. This was especially true of the higher temperatures. The fact that at the temperatures of 97° and 90° F a larger percentage of the lemons showed infection than at 82° F, which has been given as the approximate optimum for the development of Alternaria in lemons, might be taken to indicate that 82° F is really not the optimum. However, the slightly greater percentage of infection at the highest temperatures, which are above the optimum for the growth of the fungus, may have been due to a weakened condition of the lemon tissues resulting from exposure to these high temperatures. Examination showed that the fungus had developed much farther in the lemons held at 82° F than in those held at the higher temperatures.

2. Averaging the data for all temperatures, the uninoculated silvers had only one per cent less infection than the inoculated, while in the light greens the two groups of uninoculated had one and four per cent

respectively more infection than the inoculated. This indicates that the Alternaria organism was already present in or beneath each lemon button when the lemons were picked and with sufficient time and favorable conditions practically all of these lemons would have shown decay without artificial inoculation.

3. The infection was approximately ten per cent greater in the silvers than in the light greens, showing, as was indicated in tables 1 and 2, that the longer the lemons are left on the trees after becoming practically mature, the sooner will they show decay by Alternaria after being placed in storage. The data in table 5 emphasize the correctness of this statement even more markedly.

GROWTH RATE OF ALTERNARIA MYCELIUM

Having found that 82° F was approximately the optimum temperature for the development of Alternaria rot in the lemons, it seemed advisable to determine next the optimum temperature for the rate of growth of Alternaria in artificial cultures. For this purpose the same constant-temperature apparatus was used but, as indicated in table 4,

TABLE 4

THE EFFECT OF DIFFERENT TEMPERATURES ON THE RATE OF GROWTH OF ALTERNARIA MYCELIUM ON AN ARTIFICIAL CULTURE MEDIUM (CORNMEAL AGAR). TRANSFERS WERE MADE ON JANUARY 6, AT 9 A.M.

Times of measurement	Diameters of mycelial discs growing at the following temperatures, °F						
	48	58	66	72	79	85	94
	cm	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
Jan. 8, 9 a.m.	0.4	0.8	1.6	1.6	2.1	2.0	0.7
Jan. 9, 9 a.m.	0.5	1.7	2.3	2.8	3.3	3.0	0.9
Jan. 10, 9 a.m.	0.9	1.9	3.0	3.6	5.2	4.2	1.2

the range of temperatures was a little lower than in the preceding experiment. Sterilized Petri dishes and glucose-potato and cornmeal agar were used. A very small droplet of water containing Alternaria spores in suspension was placed on the center of the culture medium in each Petri dish by means of a small, sterilized platinum loop. An equal number of dishes of the two kinds of culture media was then placed in each of the different constant-temperature compartments. It is characteristic of Alternaria mycelium, when grown on culture media of this kind, to grow approximately equally in a plane in all directions from the original point of inoculation, thus making a disc-shaped mat. The rate of growth was determined by measuring at

intervals the diameter of each disc of mycelium. While the character of the mycelium on the glucose-potato agar was a little different from that on cornmeal agar, the rate of growth on the two was so nearly the same that only the averages for the latter are given (table 4).

The data show (*a*) that the optimum temperature for the growth of Alternaria on these two artificial culture media is approximately the same as in lemon fruits (82° F), and (*b*) that Alternaria will grow slowly at least, at temperatures as low as 48° and as high as 94° F. These results agree very well with the data obtained on the development of Alternaria in lemon tissues (table 3).

RESISTANCE OF ISOLATED ALTERNARIA SPORES AND MYCELIUM

Tests on the resistance of Alternaria spores and mycelium to mercuric chloride and to hot water were made, preliminary to testing the efficiency of various sterilizing solutions for the prevention of Alternaria rot in lemons.

Mercuric chloride.—Pure cultures of Alternaria, isolated from lemons, were grown on nutrient agar in Petri dishes. As soon as the surface of the medium became well covered with mycelium and spores, small pieces of the agar (approximately $\frac{1}{16} \times \frac{1}{16} \times \frac{1}{8}$ inch) were removed and placed in small glass funnels lined with filter paper. The funnels were then immersed in a solution of 1 part mercuric chloride to 1000 parts of water and kept at a temperature of 75° F. After periods of 2, 4, 6, 8, and 10 minutes respectively a given number of the funnels was removed from the sterilizing solution and washed repeatedly with sterile distilled water for half an hour to remove all traces of mercuric chloride. The pieces of agar, bearing the spores and mycelium, were then transferred to fresh culture medium in Petri dishes and incubated at 77° F. Small pieces of the original culture which had not been sterilized were washed with sterile distilled water and similarly incubated to serve as checks.

All of the unsterilized cultures showed the characteristic development of Alternaria while in all of the treated cultures growth was entirely lacking.

The results of this experiment show that Alternaria spores and mycelium, grown on artificial culture media, may be killed by immersing them for only two minutes in a solution of 1 to 1000 mercuric chloride and water, kept at 75° F.

In another experiment mature fruits and twigs were brought to the laboratory and their surfaces washed with a brush dipped in sterile distilled water. Portions of the water were then filtered through

filter paper in the small funnels used in the previous experiment. Microscopical examination showed the presence of an abundance of Alternaria spores and some bits of mycelium. The mycelium, where present, was composed of small, irregular pieces such as one would expect to find produced under adverse conditions. The process of sterilization was the same as in the preceding experiment. After washing, small masses of the spores and mycelium were scraped from the filter papers, transferred to culture dishes and incubated at 77° F.

The results were similar to those of the preceding experiment. The unsterilized checks showed 100 per cent growth but there was no growth in any of the cultures where the spores and mycelium had been exposed to the sterilizing solution for periods of 2, 4, 6, 8, or 10 minutes. Evidently the Alternaria organism whether obtained from artificial or natural cultures may be killed by immersion for a period of only two minutes in a solution of 1 part mercuric chloride in 1000 parts of water at a temperature of 75° F.

Hot water.—Since hot water at a temperature of 115 to 125° F is commonly used in the lemon packing houses as a means of controlling brown rot (*Pythiacystis citrophthora*), it seemed desirable to determine the effects of hot-water treatment on Alternaria. This test was conducted in the same manner as the two preceding ones, except that hot water (116° F) was used as the sterilizing medium instead of mercuric chloride.

In this test all cultures showed 100 per cent Alternaria infection. It was interesting to note, however, that the treatment retarded the rate of growth, especially in the cultures that had been exposed for ten minutes.

RESISTANCE OF ALTERNARIA SPORES AND MYCELIUM WHILE ADHERING TO THE SURFACE OF LEMON FRUITS AND TWIGS

The mercuric chloride solution was tested next on spores and mycelium in contact with the surface of the tissues. Thin strips of peel taken from mature lemon fruits were first used. Microscopical study showed that the mycelium had not penetrated the surface layer of cells. The strips, approximately ⅛ inch thick, were cut into pieces about ¼ inch square. Forty of these pieces were put into each of five cheese-cloth bags, and vacuumized in a flask of water for one hour to remove all air that might prevent the sterilizing solution from coming into contact with the spores or mycelium. Upon removal from the vacuum flask four of the bags were immersed in the sterilizing

solution. They were removed successively from this solution at intervals of 2, 4, 6, and 8 minutes. After thorough washing to remove the mercuric chloride, the pieces of peel were plated on a culture medium and incubated at 77° F. The pieces of peel from the fifth bag were not sterilized but were cultured in the same way as the others, to serve as checks. The sterilizing solution was the same as before and was used at 73° F.

The percentages of Alternaria appearing in these cultures at the end of eight days were as follows: checks, 35 per cent; immersed 2 minutes, 18 per cent; 4 minutes, 13 per cent; 6 minutes, 8 per cent; and 8 minutes, none.

The second part of the test was similar to the first part except that portions of twigs were used instead of pieces of peel, and they were immersed in the sterilizing solution for only two minutes. The portion of the twig used was about ¼ inch of the pedicel, the portion of the stem just below the button. This part of the twig is free from bracts, which might cover the spores, and to the unaided eye it appears to be entirely smooth. Sixty-four of these pieces were treated and then cultured as in the first part of the test.

As in the case of the peel it was found that a two-minute exposure to the sterilizing solution was not sufficient to kill all of the Alternaria. After one week in the incubator 19 per cent of the pieces showed a growth of Alternaria.

The results of both parts of the preceding test indicate rather strongly that even though an exposure of isolated spores or mycelium to a sterilizing solution for a given period will kill them it is not safe to conclude that a like exposure will kill them when they are in contact with, but not embedded in, the tissues which at least in some cases they may enter later. Had not previous examination shown that such was not the case one might have concluded that the reason for the growth of Alternaria in connection with some of the pieces of peel or twig was because the mycelium had already penetrated the tissues and was out of reach of the sterilizing solution.

STERILIZATION OF LEMON FRUITS AS A MEANS OF PREVENTING ALTERNARIA ROT

The next step in the study was an attempt to at least minimize if not entirely prevent this decay by sterilizing the fruits. The plan followed was (1) to use mercuric chloride in the wash water in the packing house, (2) to use ''brogdite''[7] in the wash water in the

[7] Brogdite is a commercial sterilizing medium that is being used in some packing houses to prevent decay caused by blue and green molds.

packing house, (3) to remove the buttons, and (4) to test the efficiency of various sterilizing solutions in the laboratory, under controlled conditions, provided the other methods did not prove to be effective or practicable.

1. Tests were made in two packing houses. In one house five boxes of silver lemons and twenty-two boxes of green lemons were washed in each of the following: (*a*) water at approximately 95° F, (*b*) mercuric chloride sterilizing solution at 60° F, and (*c*) the same solution at 110° F. The lemons were retained in the solution approximately five minutes, then rinsed in the usual manner and put into storage.

The fruit in the other packing house (three boxes of yellows and five boxes each of silvers and greens) was treated in the same manner. The fruit in both houses was examined at intervals for three and a half months.

The results of the tests in these two packing houses indicate that mercuric chloride can not be considered an efficient sterilizing medium for the prevention of Alternaria rot in lemons. Apparently the cold mercuric chloride solution had no controlling effect whatsoever, and while the results with the hot solution indicated a slight degree of control it was considered to be more of a retarding than an actual sterilizing effect.

2. In the test with brogdite eight boxes of yellow lemons (1700 fruits) were divided into four lots of two boxes each. The first lot was washed in water at approximately 113° F and the second, third, and fourth lots were immersed in brogdite solution of the same temperature for 5, 5, and 10 minutes respectively, after which they were rinsed in cold water. The strength of the solution for the second lot was sixteen ounces of brogdite to a gallon of water, and for the third and fourth lots, twenty-four ounces.

By the end of two months 2, 4, 16, and 8 per cent respectively of Alternaria rot had appeared in the four lots. While only one test was made with this solution at this time the results indicated that it would be useless to repeat it. That more Alternaria rot appeared in the treated than in the untreated lemons indicates that the treatment produced or hastened some change in the lemon tissues which permitted a more rapid invasion by the fungus. It should be said, however, that there was no external indication of injury due to the treatment.

3. In each of four packing houses, six boxes of yellow lemons[8] and six boxes of green lemons were given the usual treatment of washing

[8] Although these lemons were yellow they were not classed as "tree-ripes." They had been prematurely colored by the winds and comparatively low temperatures prevailing during November and December.

and sorting. Three boxes of yellows and three of greens were placed under storage conditions. The remaining three boxes each of yellows and greens were sweated to such an extent as to cause at least most of the buttons to drop. This was done to determine whether the removal of the buttons would cause a reduction of the amount of Alternaria rot. In two of the houses the sweating was done with ethylene gas and in the other two with oil stoves. The sweating periods in packing houses 1 to 4 were 8, 8, 7, and 8 days respectively. The sweating caused no visible injury to the fruit. At the end of the sweating period any buttons still attached were removed by hand. These boxes of lemons were then placed in the storeroom beside the untreated checks. All boxes of lemons were examined at intervals for four months after the last boxes were placed in storage. The total number of lemons in each lot showing Alternaria rot within that period is shown in table 5. The exact number of lemons used was not determined but there were from 375 to 400 in each box.

TABLE 5

THE AMOUNT OF ALTERNARIA ROT IN LEMONS AS INFLUENCED BY THE REMOVAL OF THE BUTTONS. THREE BOXES IN EACH LOT.

Packing house	Sweated, buttons removed		Checks, buttons not removed	
	Yellow	Green	Yellow	Green
	Number of lemons showing Alternaria rot			
1	157	1	223	2
2	55	13	30	3
3	20	3	21	2
4	46	49	118	2

The results indicate that the removal of the buttons from the lemons is not a feasible method for the control of Alternaria rot. By referring to the table it may be seen that in packing house No. 1 there was apparently 30 per cent control and in house No. 4, 20 per cent, but in house No. 3 there was no indication of control, and in house No. 2 there was 51 per cent more Alternaria rot in those lemons which had the buttons removed than in the checks.

A similar test was later conducted in the laboratory. In this case all of the lemons were silvers and the buttons were removed by hand rather than by sweating. The fruit was brought directly from the grove and stored in field boxes in a basement room of the laboratory where the temperature did not vary more than four degrees, remaining

for the entire time of the test (December 21 to March 5) at approximately 70° F. This temperature, although below the optimum, is very favorable for the growth of Alternaria. The humidity of the room was such that by the end of the test the lemons showed about the same amount of shrinkage as would have occurred under the usual storage conditions in the packing house.

On January 8 none of the fruits showed decay, but the temperature and humidity had been favorable for the germination of Alternaria spores that were attached to the buttons and to the surface of the fruits. Small mats of mycelium could be distinguished very easily with the unaided eye. The appearance of these mats of mycelium, as they formed on the buttons in this and other tests and as they often occur under packing house conditions, is shown in plate 2, figures A and B. It was especially interesting that more mats of mycelium could be seen in the button pits of those lemons from which the buttons had been removed than could be seen in connection with the buttons that remained attached to the lemons. This condition gives further evidence that removal of the buttons does not necessarily remove all chances of infection. Data to be presented farther on will show that, although the button be removed, there are left in the crevices in the tissues under the button a sufficient number of spores and bits of resistant mycelium to cause infection as soon as conditions are favorable.

By March 5, sixty-three cases of Alternaria rot had been found in those lemons from which the buttons had been removed, and only six in the checks, to which the buttons remained attached. These results are comparable to those in packing house No. 2, as shown in table 5. It may be of interest to note here that although the buttons in this test were forcibly removed by using a clipper, such as is used in picking lemons, only eight lemons in the entire lot showed a growth of Penicillium.

Some of the reasons why removal of the buttons may favor infection by Alternaria are (*a*) the removal of the buttons may weaken the tissues, thus making them more susceptible to the attack of the fungus, (*b*) the evaporation from the exposed tissues may make conditions more favorable for the growth of the fungus, and (*c*) this excessive evaporation may produce changes in the lemons which make them more susceptible to attack.

Although only six lemons in the checks showed external signs of Alternaria rot further examination disclosed the fact that a large percentage of them were in the early stages of infection and would soon have become decayed. This was determined by taking thirty

lemons at random from among the checks. A thin slice was cut from the stem end of each lemon in a manner such that the knife passed just below the juncture of fruit and button, thus removing all crevices which might be harboring spores or mycelium. A small disc of tissues was then taken from each lemon immediately below the original point of attachment of the button, and cultured to determine the number that were infected. Twenty-one (70 per cent) of the thirty discs showed an abundant growth of Alternaria.

The attempt to control Alternaria rot was continued by immersing lemons in sterilizing solutions after the buttons had been removed. In the first part of the test silver lemons that had just been picked and washed were brought from three packing houses. The buttons were removed from half of each lot and both lots were immersed for five minutes in mercuric chloride of the usual strength, at 65° F. They were then put into glass containers and placed on tables in the laboratory where the temperature ranged from 54° to 72° F. Observations were made at intervals for a period of five months. The comparative amounts of Alternaria rot appearing in the different lots during this time were: from house No. 1, 8 per cent in those without buttons and 17 per cent in checks; house No. 2, 67 per cent in those without buttons and 68 per cent in checks; and from house No. 3, 8 per cent in those without buttons and 67 per cent in checks.

The second part of the test was like the first part except that only one lot of lemons was used and the sterilizing solution was copper sulphate ($\frac{1}{50}$ of 1 per cent, as commonly used in lemon packing houses). The results of this part of the test showed 51 per cent of Alternaria rots in those lemons from which the buttons had been removed and 57 per cent in the checks.

The results recorded in the two preceding paragraphs indicate that it would be impracticable to attempt to control Alternaria rot in lemons by sterilization, even after the buttons have been removed; at least this is true for the two sterilizing solutions used in this test.

ALTERNARIA IN THE LEMON TISSUE UNDER THE BUTTONS

The object of this test was to determine the percentage of fruit infection after different periods of storage in different packing houses. The lemons chosen were green or silver when picked. Some of them were tested at once but most of them had been kept in storage for from one to three months (see table 6). In order to reduce to a minimum the possibility of error through contamination, the different lots of lemons were immersed for five to ten minutes in mercuric

chloride solution at 115° to 120° F. After drying, the stem end of each lemon was sliced off just below the juncture of button and fruit. A cork borer was then inserted into the lemon so as to include the tissues just below the point of attachment of the button. A second cut was now made, parallel to the first, thus freeing a single disc of tissue from each lemon, approximately 3/16 inch thick and 1/4 inch in diameter. All operations were performed in an inoculation cage and with sterilized instruments. The discs of tissue, thirty from each lot of lemons, were cultured on an artificial medium in Petri dishes and incubated at 77° F. The percentages of discs from each lot of lemons which showed infection with Alternaria are shown in table 6.

TABLE 6

DEVELOPMENT OF ALTERNARIA FROM DISCS OF TISSUE TAKEN FROM BENEATH THE BUTTONS OF LEMONS THAT HAD JUST BEEN PICKED OR HAD BEEN KEPT IN STORAGE.

Color of lemons when picked	Packing house	Days lemons were held in storage before discs were cut	Percentage of discs from which Alternaria developed
Green	3	56	3.3
	1	61	6.7
	2	61	6.7
	4	64	0.0
	3	84	10.0
	2	92	0.0
	4	93	0.0
	1	99	3.3
Silver	6	2	0.0
	7	2	0.0
	8	2	0.0
	10	2	0.0
	11	2	0.0
	10	22	0.0
	3	40	33.3
	6	49	0.0
	9	50	3.3
	8	55	0.0
	11	55	50.0
	3	59	0.0
	4	60	6.7
	5	75	70.0

The results of this test may be summarized as follows:

1. Of the 240 discs taken from lemons which were green when picked, an average of only 3.8 per cent developed a growth of Alter-

naria. Some of the lots that had been stored for three months showed less decay than some of those stored only two months.

2. Of the 270 discs taken from the nine lots of stored lemons which were silver when picked, an average of 18.1 per cent developed a growth of Alternaria.

3. Of the 150 discs taken from the five lots of silver lemons two days after picking none showed infection.

These data would indicate (*a*) that within certain limits (see table 2) the higher the temperature the sooner the Alternaria fungus will pass from the buttons into the lemon tissues and cause them to decay, (*b*) that the more mature the fruit when picked the sooner it will become decayed, (*c*) that the general vitality of the fruit is a very important factor (e.g., see table 6, packing house 3, which shows 33.3 per cent infection in the silvers held 40 days and no infection in those held 59 days), and (*d*) that the Alternaria fungus does not usually pass from the buttons into the silver and green lemons until after they have been picked and have begun to lose their vitality.

The relations mentioned in (*d*) of the preceding paragraph do not hold true for lemons that have become tree-ripe before picking. Several tests similar to the preceding one, have shown that in a comparatively large number of cases the fungus has passed from the button into the tissues beneath by the time the tree-ripe lemons are picked. This is especially true during the warmer months.

STERILIZATION OF LEMON BUTTONS

Buttons from mature lemons.—The tests already reported in this paper have indicated that immersing the lemons, either with or without the buttons, in mercuric chloride or copper sulphate solutions of the strengths commonly used, does not control Alternaria rot. For this reason an effort was made to find some method of sterilizing detached buttons, since in this way much larger numbers could be tested and the results much more quickly determined. It was hoped that by this method some medium might be discovered which could safely be used to sterilize the buttons while they were still attached to the lemons.

The methods employed in this test are indicated in table 7, which is a general summary of the results of many trials, with most of the details omitted. Where $HgCl_2$ was used the strength of the solution was always 1 to 1000.

TABLE 7

THE EFFECTIVENESS OF CERTAIN SOLUTIONS IN STERILIZING DETACHED LEMON BUTTONS, AS INDICATED BY THE PERCENTAGE OF BUTTONS SHOWING INFECTION ON ARTIFICIAL CULTURE MEDIA

Lot	Solution and manner of application	Treated; immersed in sterilizing solution				Checks; immersed same time in cold water at room temperature			
		Number buttons	Alternaria	Miscellaneous*	Sterile	Number buttons	Alternaria	Miscellaneous*	Sterile
			Per cent	*Per cent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*
1	Tap water, 116° F, 5 min.	90	11	89	0	56	100	0	0
2	$HgCl_2$, 2 min., 65° F; then water 113° F, 5 min.	40	8	85	7	30	100	0	0
3	$HgCl_2$, 77° F, 5 min.	80	60	12	28	50	100	0	0
4	$HgCl_2$, 65° F, 7 min.	330	57	28	15	120	88	10	2
5	$HgCl_2$, 65° F, 10 min.	210	55	14	31	100	56	44	0
6	$HgCl_2$, 65° F, 30 min.	111	26	6	68	60	88	12	0
7	$HgCl_2$, 118° F, 5 min.	340	24	6	70	184	99	0	1
8	H_2O in vacuum, 68° F, 30 min.; then $HgCl_2$, 65° F, 5 min.	196	64	4	32	80	85	15	0
9	95% alcohol, 2 min.; then $HgCl_2$ in 50% alc., 10 min.	40	58	12	30	40	95	5	0
10	95% alcohol, 2 min.; then $HgCl_2$ in 50% alc., 20 min.	150	35	18	47	50	100	0	0
11	$CuSO_4$ (1/50 of 1%), 65° F, 5 min.	80	100	0	0	74	99	0	1
12	$CuSO_4$ (1/50 of 1%), 115° F, 5 min.	50	38	62	0	26	100	0	0
13	Brogdite, 16 oz. per gal., 118° F, 20 min.	150	88	12	0	50	86	14	0
14	Semesan† (1 to 400 in water), 70° F, 30 min.	116	86	12	2	50	100	0	0
15	Semesan (1 to 400 in water), 70° F, 1 hr.	131	76	8	16	50	100	0	0
16	Formalin (1 to 10,000 in water), 61° F, 5 min.	30	100	0	0	30	93	7	0
17	Formalin (1 to 10,000 in water), 113° F, 5 min.	30	67	30	3	.....	.....	.....	.....
18	Formalin (1 to 2,000 in water), 61° F, 5 min.	30	97	3	0	.....	.....	.....	.....
19	Formalin (1 to 2,000 in water), 113° F, 5 min.	30	33	67	0	.....	.....	.....	.....
20	Formalin (1 to 1,000 in water), 61° F, 5 min.	30	97	3	0	.....	.....	.....	.....
21	Formalin (1 to 1,000 in water), 113° F, 5 min.	30	17	83	0	.....	.....	.....	.....
22	$Hg(CN)_2$ (1 to 4,000 in water), 65° F, 5 min.	30	97	3	0	30	96	4	0
23	$Hg(CN)_2$ (1 to 2,000 in water), 65° F, 5 min.	30	87	13	0	.....	.....	.....	.....
24	$Hg(CN)_2$ (1 to 1,000 in water), 65° F, 5 min.	30	80	20	0	.....	.....	.....	.....
25	$Hg(CN)_2$ (1 to 1,000 in water), 113° F, 5 min.	30	37	63	0	.....	.....	.....	.....

* In the columns headed "miscellaneous" were placed those buttons which showed some other infection than Alternaria, usually Mucor, Penicillium, Colletotrichum and bacteria. However, careful examination indicated that many of the buttons listed in this column were infected with Alternaria but that it had been outgrown by one, or possibly more, of these organisms.

† Semesan is a commercial compound used for the sterilization of seeds.

The conclusions may be summarized as follows:

1. None of the solutions used can be considered to be efficient for the prevention of the development of Alternaria in detached lemon buttons. Higher concentrations, higher temperatures and longer periods of exposure would doubtless effect greater degrees of sterilization of the detached buttons, but these would have to be so great that the treatment would be impracticable under packing-house conditions and, furthermore, would result in injury to the fruit.

2. Some of the solutions appeared to have more of a retarding effect on Alternaria than on some of the other organisms such as Mucor, Penicillium, Aspergillus, Colletotrichum and bacteria, and as a result the largest percentage of infection is shown in the column headed ''Miscellaneous'' (see lots number 12 and 21). That this effect was due to retardation rather than killing of the Alternaria is at least indicated by the results from lots 1 and 2 in which there was a marked reduction in the percentage of Alternaria but no sterilization in lot 1 and only 7 per cent in lot 2. Similarly, in a preceding test hot water alone was used on pure cultures of Alternaria and the treatment failed to kill the fungus but produced a very noticeable retardation in its development. From these results it appears that the Alternaria in such cases as lots 1, 2, 12 and 21 was not actually killed but that its rate of growth was temporarily inhibited or retarded to such an extent that it was overgrown and further retarded by some of the other organisms, and could not be detected when the cultures were examined. Repeated tests have shown that hot water is not a good sterilizing medium for the control of Alternaria rot, since its retarding effect is apparently only temporary.

3. An examination of the data pertaining to the checks shows that a very large percentage of the buttons was infected with Alternaria. It seems very probable that many of those listed in the miscellaneous column were infected with Alternaria but that it was outgrown by other organisms. In lot 5, for example, 44 per cent of the buttons are listed in the miscellaneous column. These buttons were taken from lemons which, while practically mature, were on the trees during the freeze of 1923. These particular lemons, when picked a few weeks later, showed very slight, if any, visible injury, but it is of interest to note that in the checks, 44 per cent of the buttons from these lemons showed a predominant growth of Colletotrichum. Apparently the low temperature had produced changes in the tissues that made them more favorable for the growth of Colletotrichum than for that of Alternaria. This possibility is also proved by the fact that much more Colletotrichum rot is found in lemons following a freeze than is found

under the usual conditions. With further reference to lot 5, it would seem that mercuric chloride, as here used, is a better sterilizing agent for Colletotrichum than for Alternaria.

The buttons from green and silver lemons are not reported separately in table 7 since it appears that the vitality of the tissues rather than their age is the principal factor determining the percentage of infection. Apparently the vitality of the tissues governs the depth of penetration of the Alternaria and in turn this factor at least partially determines the percentage of sterilization.

Buttons from young lemons.—In February buttons were taken from immature lemons, of the average diameters shown in table 8. Before the buttons were placed in the culture dishes they were soaked in mercuric chloride solution of the usual strength for two minutes at 65° F.

TABLE 8

PERCENTAGES OF ALTERNARIA, OR OTHER INFECTION, FOUND IN BUTTONS TAKEN FROM YOUNG LEMONS OF DIFFERENT SIZES.

Number of buttons	Average diameter of lemons	Alternaria infection	Miscellaneous infection	Sterile
	Inches	*Per cent*	*Per cent*	*Per cent*
50	$1\frac{12}{16}$	80	8	12
50	$1\frac{1}{16}$	84	6	10
50	$\frac{9}{16}$	70	24	6

The data in table 8 indicate that a very high percentage of the buttons, even from lemons that were only $\frac{9}{16}$ of an inch in diameter, were at least potentially infected with Alternaria. It should be borne in mind, however, that these buttons had been immersed in the sterilizing solution for only two minutes and that at least not all spores adhering to the surface, to say nothing of those which may be protected in crevices, are killed by such an exposure.

In another test the larger crevices were eliminated by discarding the enlarged central portion of the button, using only the comparatively thin outer layer of tissues which compose the calyx ring. The sterilizing conditions were also made more rigorous. The calyx rings were first soaked for two minutes in 95 per cent alcohol, to ensure a complete wetting of the surface, and then for five minutes in 1 part mercuric chloride to 1000 parts of 50 per cent alcohol. In this test the calyx rings from lemons of two sizes were used. The larger lemons were approximately $1\frac{1}{4}$ inches in diameter while the smaller ones were picked just after the petals had fallen and were approximately $\frac{1}{8}$ inch in diameter.

The results of this test showed 90 per cent Alternaria infection in the calyx rings from the larger lemons and none in those from the smaller ones. In the case of the older calyx rings this seems to indicate either that the sterilizing agent did not kill all surface spores or that the fungus had entered the tissues sufficiently far to be out of reach of the sterilizing solution as applied. The results of a microscopical study which will be reported later in this paper, indicate that the latter explanation is the more probable. This conclusion is also confirmed by the fact that the controls for the smaller sizes showed 60 per cent Alternaria growth while those which had been immersed in the sterilizing solution showed none. This result indicates that the treatment was sufficient to kill surface-adhering spores. Some portions of the calyx rings from some of the smaller lemons had begun to turn brown as if infection had started, but if this was the case the penetration of the mycelium was so nearly superficial that its further development was prevented by the sterilization process.

The high percentage of negative results obtained in attempting to sterilize detached lemon buttons seem to indicate that attempted sterilization of attached buttons, without injury to the fruit, is impracticable.

SPRAYING TO CONTROL ALTERNARIA ROT

Experiments were conducted with a view to the control of Alternaria rot by means of orchard spraying. In one grove twelve rows of twenty trees each were sprayed with Bordeaux (3–3–50) in April just following the heaviest spring set of fruits. Six of these rows were sprayed again in September following the heaviest fall set. In a second grove 147 trees were sprayed in April, 72 of which were sprayed again in November.

Cultures of buttons taken from the sprayed trees failed to reveal any beneficial effect from the treatment.

During the course of the preceding spraying test a second and more carefully conducted one was made in a different grove. Seven hundred twenty lemon blossoms that had just opened and 500 young lemons about ¼ inch in diameter, were individually sprayed with Bordeaux mixture (4–4–50) by a hand atomizer. Care was taken to see that all parts of the blossoms, the small fruits, the calyx cups and the adjacent parts of the stems were thoroughly wet by the spray mixture. Seventy-seven days later the sprayed lemons, and controls of a similar size, were picked and the buttons removed and cultured to determine the relative percentages of Alternaria infection in the

three lots. When picked, the lemons from the sprayed blossoms had attained an average diameter of $^{25}\!/_{32}$ inch while the fruits which were $1/4$ inch in diameter when sprayed had become $1^{3}\!/_{32}$ inches in diameter. Buttons were taken from only one lot of controls and their average diameter was approximately that of the larger of the two sprayed lots. The percentages of infection appearing in the three lots of buttons were (*a*) in those taken from the fruits from the sprayed blossoms, 57 per cent Alternaria, 43 per cent miscellaneous infection and none sterile, (*b*) in those from the fruits $1/4$ inch in diameter when sprayed, 70 per cent Alternaria, 30 per cent miscellaneous and none sterile, and (*c*) in the controls, 68 per cent Alternaria, 32 per cent miscellaneous and none sterile.

The results of the two preceding tests, coupled with the fact that there is an almost continuous setting of young fruits on lemon trees, would indicate that there is little hope of satisfactorily controlling or even materially reducing the percentage of Alternaria rot in lemons by means of orchard spraying.

MICROSCOPICAL STUDY OF THE LEMON FRUIT AND BUTTON TISSUES INFECTED WITH ALTERNARIA

The results of the tests reported thus far in this paper show the comparative inefficiency of the usual methods as means of controlling Alternaria rot of lemons. The inefficiency of these methods suggests that spores or resistant mycelium were protected from the sterilizing solutions by being in depressions or crevices in the button or in the fruit beneath it, or that the mycelium had already penetrated the tissues of the button or lemon and was out of reach of the sterilizing solutions. To determine whether such could be the case, microscopical studies were made of the tissues in question. For this purpose lemon buttons and portions of the lemon tissues just beneath the buttons were collected, killed, fixed, embedded, sectioned, stained with differential stains, and the sections studied microscopically to detect the presence or absence of spores or mycelium on or in these tissues.

Weak chrom-acetic acid seemed to give better results than any of the several other killing solutions tried. Of the several different stain combinations commonly used for differentiating fungus mycelium and host tissues, such as Pianeze, Delafield's Haematoxylin and Eosin, and Mazdala Red and Light Green, the last gave the best results.

The age of the lemons from which the buttons and other tissues were taken is indicated by the fact that all of them had been picked previously for commercial purposes. The color of the lemons at time

of picking and their treatment during the interval between picking and the time at which the buttons and lemon tissues were selected for this study is stated in the legends of plates 2 and 3.[9] Figures 1 to 12, plates 2 and 3, are photomicrographs of portions of some of the sections of the stained tissues. The magnifications were the same for all of these figures.

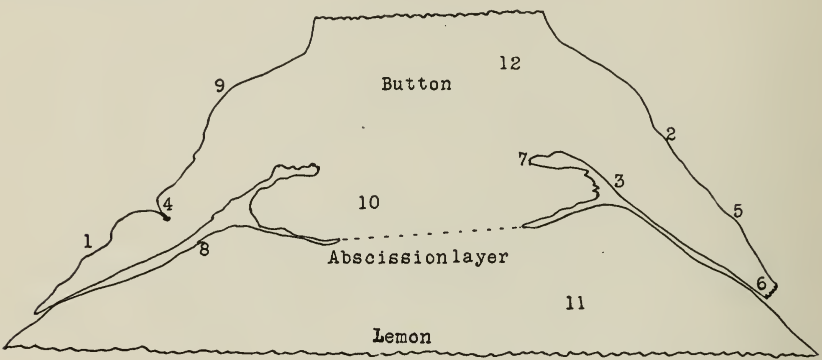

Fig. 1.—Outline of a projection of a microscopical section which shows the configuration of a button and its attachment to the fruit. The numerals placed on different portions of the outline designate the approximate regions of the buttons or fruits from which the sections were taken which are figured in plates 2 and 3.

The microscopical study of the tissues in question indicates:

1. The ineffectiveness of sterilizing agents seems to be due to the presence of spores and mycelium in depressions or crevices where they are inaccessible to the sterilizing solution, and to penetration of mycelium into the button tissues where it is out of reach of the sterilizing solution (pl. 2, fig. 4, and pl. 3, figs. 5, 6, 7, and 8).

2. In some cases spores and resistant mycelium were found attached to the surface of the button but with no evident attempt at penetration (pl. 2, fig. 2). In other cases apparently the spore had germinated on the surface and had made an unsuccessful attempt at penetration, but had produced certain changes in the button tissue directly beneath (pl. 2, fig. 1). In most cases where the fungus mycelium had gained entrance the tissue of the button had formed a resistant or semi-resistant layer of tissue in advance of the invasion of the fungus (pl. 2, figs. 1, 3, and 4, and pl. 3, figs. 6 and 7). The cells composing the resistant layer are or at least very closely resemble young cork cells. They are almost or entirely free from protoplasmic contents and their exterior walls are thicker than their lateral or inner walls.

[9] To visualize the region of the buttons or lemons from which figures 1 to 12, plates 2 and 3, were taken, frequent reference should be made to text figure 1.

A cork cambium is very evident in most cases and some of the pre-existing cells which were pushed off by the newly-formed cork layers are found intermingled with the mats of spores and mycelium on the surface (pl. 2, figs. 3, and 4, and pl. 3, fig. 7). In some cases the fungus seems to have been able to pass this barrier imposed by the host, and as many as three successive layers of cork tissue have been formed at intervals in the effort to check the invasion of the fungus.

3. In some cases the invasion of the fungus was so rapid that apparently the host tissues did not have time to form the resistant layer of cork cells until considerable progress had been made by the fungus. Plate 3, figure 5 shows such a case which occurred under field conditions (compare pl. 2, figs. 1 and 3, and pl. 3, figs. 6 and 7). Under field conditions the vitality of the tissues of the button and lemon fruit is usually at its maximum while conditions for the germination and growth of the fungus are usually unfavorable. But when the lemons have been picked and stored the conditions are reversed and the fungus makes much more rapid progress. The result of an exaggerated case of this reversal of conditions is shown in plate 3, figures 9 and 12. In this case buttons from green lemons were placed in Petri dishes containing an artificial medium very favorable for the growth of the fungus. Had these lemons, with their buttons attached, been placed under usual storage conditions it probably would have been at least three months before the fungus would have progressed through the buttons and into the lemons to such an extent as to cause visible signs of decay. Under the favorable conditions in the Petri dish the fungus penetrated all portions of the buttons in eight days and so modified their internal structure that the individual cells had lost their identity, except in the case of the more resistant wood cells. In the prepared sections the interior of the buttons appeared to be almost a homogeneous mass with the mycelium penetrating all portions of it (pl. 3, fig. 12). The figure just mentioned should be compared with figure 11 in the same plate, noting comparative diameters of the mycelial threads and the nature of the host tissues. Figure 11 represents the conditions in a button that had been taken from a lemon kept in the sweatroom for ten days and then held in storage for another ten days.

4. As a rule the fungus mycelium has not passed from the button down into the fruit tissues at the time of picking. Exceptions to this rule may be found in cases where the lemons have become over-ripe before picking or where they have been exposed to excessively high or low temperatures. Apparently the penetration does not take place until the lemon tissues have become "weakened" (changed physio-

logically) to a certain extent. It seems also that any environment which produces a weakened condition in the fruit, such as a comparatively high storage temperature, also produces conditions favorable for rapid growth of the fungus. The combination of these two effects results in a more or less rapid decay of the fruit.

5. The microscopical study further showed why very many of the stored lemons may be subject to the attack of Alternaria even though the buttons have been removed and the lemons soaked in a sterilizing solution before being placed in storage. Those who are familiar with the early stages of development of the lemon fruit know that after the petals have fallen there is left behind the calyx cup, in the center of which the young fruit is attached. This calyx cup makes a suitable receptacle for catching Alternaria spores. As the fruit enlarges, the edges of the calyx cup come in contact with the fruit and the Alternaria spores are thus entrapped. In this manner some of the spores, or bits of resistant mycelium from spores that germinated and grew for only a short time, may easily find lodgment in some of the minute depressions or crevices in the fruit under the button. Again, as the fruit enlarges it no longer maintains an upright position but hangs downward and in many cases portions of the edges of the button lose their close contact with the fruit. This condition affords another chance for the entrance of spores into the space between the button and fruit. These spores or resistant bits of mycelium are not reached by the sterilizing solutions under the usual methods of application. Plate 3, figure 8 shows two small pieces of resistant mycelium in a microscopical crevice in the surface of a lemon just beneath the button.

6. In describing the characteristic appearance of the lemon tissues beneath the button, in the early stages of infection with Alternaria, it was stated in a previous section of this paper that the individual vascular bundles appeared to be infected first, as indicated by the fact that they are first to show discoloration. The microscopical study has shown that initial infection probably takes place first in the phloem, or in the bundle sheath, and passes from there into the surrounding parenchyma or into and among the water-conducting vessels and other xylem elements. Plate 3, figure 10 shows mycelium among the wood cells and medullary rays and in a water conducting vessel. No evidence was found to indicate that the Alternaria mycelium enters the water-conducting vessels of the button, while the lemon is still attached to the tree, to such an extent as to restrict the passage of water.

DISCUSSION OF CONTROL MEASURES

The commonly used methods of spraying and sterilization appear to be in a large measure ineffective as a means of controlling Alternaria rot of lemons. The ineffectiveness in the case of spraying is very largely due to the fact that new fruits are being formed almost continuously and that Alternaria spores are always present in the air and on all parts of the tree. These spores are constantly being carried to the newly formed tissues either by air currents or rain. The difficulty in sterilizing the lemon fruits after they have been picked lies in the fact that the mycelium has already entered the button tissues or that the spores are located in crevices in the button or in the lemon tissue beneath the button, so that both mycelium and spores are inaccessible to the sterilizing solutions commonly used for such purposes. Various new solutions are being tested by different workers and it is hoped that one may be found which will be able to effect sterilization without injury to the fruit.

Rogers and Earle,[10] who worked at San Pedro, Isle of Pines, on control measures for stem-end rot[11] of several types of citrus fruits, recommend that the fruits be pulled instead of clipped from the tree and that the scar on the fruit, left by detaching it from the stem, be covered with shellac. An initial test of this method proved it to be so inefficient for the control of Alternaria rot that it was not given further attention. Even were this method more or less effective it does not seem that it would be practicable under California conditions where such a large crop of lemons is produced. Winston, Fulton and Bowman,[12] working in Florida, have found that both orchard spraying and the removal of the buttons from the fruits very noticeably reduce the amount of stem-end rot in oranges and grapefruit. They have found also that pruning the dead branches out of the trees tends to reduce stem-end rot, especially where Diplodia is concerned. The almost universal presence of Alternaria spores on decaying vegetation, coupled with the fact that the new fruits are forming almost continuously, indicates that pruning would be comparatively ineffective in controlling Alternaria rot of lemons.

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[10] Rogers, J. M., and F. S. Earle. A simple and effective method of protecting citrus fruits against stem-end rot. Phytopathology 7: 361–367. 1917.

[11] Stem-end rot is somewhat similar to Alternaria rot in that the fungus enters the fruit through the stem end. It is principally caused by *Phomopsis citri* and *Diplodia natalensis*.

[12] Winston, J. R., H. R. Fulton, and J. J. Bowman. Commercial control of stem-end rot. The Florida Grower 28: 6, 26. 1923.

With reference to the removal of the buttons as a means of control, it ordinarily requires a week or more to sweat green lemons to give them the desired color. Under such conditions the buttons remain attached to the lemons. If the conditions were intensified enough during this process to cause the buttons to drop there would be the added danger of injury to the fruit and also the probability of causing physiological changes in it which would favor the growth of Alternaria. In Florida it is stated that an exposure to ethylene gas, at a temperature of 80° to 85° F and a humidity of 85 to 90 per cent for thirty-six hours will cause orange and grapefruit buttons to loosen.

At the present time and under California conditions it is not possible to prevent Alternaria rot of lemons satisfactorily, but it has been found that the losses from this disease may be materially reduced by the adoption of the following suggestions:

1. The trees should be kept as nearly as possible in a healthy condition so that the fruit will grow continuously from setting to maturity. Fruits that have experienced a set-back, due to lack of water or other cause, are weaker than if they had grown continuously and hence are more susceptible to attack by Alternaria.

2. The fruit should be picked while it has a high vitality, i.e., while it is yet silver or green in color. The Alternaria rot fungus cannot attack it as long as its vitality is high.

3. Washing the fruit in hot water materially raises its temperature. If this temperature could be reduced before the fruit is placed in storage the chances for the development of Alternaria rot would probably be reduced. It is a well-known fact that a sudden reduction of temperature, following a comparatively high one, markedly retards the development of various diseases.

4. If the fruit can be dried sufficiently to remove all free water from beneath the buttons before the fruit is placed in the storeroom, conditions are made less favorable for the development of Alternaria.

5. Minimum temperature, humidity and time of exposure should be used when sweating green fruit.

6. So far as possible, all fruits affected with endoxerosis (internal decline, blossom-end decay, etc.) should be excluded.

7. The fruit should be stored at a low temperature. The development of Alternaria rot in lemons is extremely slow at a temperature of 55° F, or lower, unless the fruit has a low vitality.

8. During the process of grading and packing, an unnecessarily long exposure of the fruit to the warmer temperature of the grading room should be avoided.

9. Before packing for shipment each lot of fruit should be carefully inspected for the presence of Alternaria rot in its early stages, i.e., before it is visible on the surface. This inspection should be made by slicing off the stem end of the lemon, just under the button. If the lemon has become infected the small bundles of tissues in the center of the cut surface will have a pinkish to light brown color. If a given lot of fruit examined in this manner shows more than an extremely low percentage of infection it should not be shipped, or if shipped it should be placed on a market where the time consumed in transit will be short and where the chances for its early consumption are good.

SUMMARY

1. Alternaria probably causes more California lemon fruits to decay than any other one fungus, with the possible exception of Penicillium.

2. Alternaria rot may be found in all lemon-growing districts of California.

3. The lemon fruits become potentially infected with Alternaria before picking, but they do not usually begin to decay until after being placed in storage.

4. Alternaria rot of lemons is caused by the fungus Alternaria; endoxerosis of lemons is not caused by a fungus but by abnormal physiological conditions. Care should be exercised not to confuse the two.

5. The temperature range most suitable for the maximum growth rate of Alternaria either in the lemon tissues or in the culture, is approximately 78° to 83° F.

6. Isolated Alternaria spores or resistant mycelium may be killed by a two-minute exposure to 1:1000 mercuric chloride, but if they are in contact with the surface of the lemons, buttons, or twigs, it appears that an exposure of at least six to eight minutes is required in order to kill them.

7. To attempt to prevent Alternaria rot of lemons by the methods commonly used for such purposes seems impracticable.

8. The removal of the buttons before placing the lemons in storage is not effective in controlling Alternaria rot, and, furthermore, such a procedure would be impracticable.

9. Although the button becomes infected while the fruit is very young and spores are also entrapped under the button, it is only in exceptional cases that the Alternaria fungus has entered the fruit tissues by the time the lemons are picked.

10. Alternaria may enter the lemon directly from the button or from spores or mycelium harbored between the button and the lemon.

11. If lemons of average vitality are stored at a temperature of 55° to 60° F, Alternaria rot will usually begin to appear in tree-ripes in from one month to six weeks, in silvers in from two to two and a half months, and in greens from three to four months.

12. Present methods of orchard spraying appear to be both unsuccessful and impracticable for the prevention of Alternaria rot of lemons.

13. A microscopical study of the button and lemon tissues concerned has shown (*a*) attempts at sterilization were unsuccessful because spores and resistant mycelium were located in depressions or crevices where they were inaccessible to the sterilizing solution and because in most cases some of the mycelium had already penetrated the button tissue where it too was out of reach of the sterilizing solution, (*b*) the button tissues retard the advance of the fungus, after it has once entered, by the formation of a corky layer, (*c*) as the Alternaria enters the fruit it first follows the bundle sheath and then it passes into the parenchyma and into the water-conducting vessels and other wood elements, and (*d*) crevices and depressions in the lemon tissues under the button harbor spores and resistant bits of mycelium which, though the button be removed, may produce infection as soon as conditions become favorable.

14. Certain methods of picking, storing, etc., will materially reduce the amount of loss due to Alternaria rot.

It may be said in conclusion that while Alternaria is the organism which directly produces the decay under discussion in this paper, it is realized that some of the other organisms, often found associated with the Alternaria, may contribute toward making conditions more favorable for its development.

ACKNOWLEDGMENTS

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PLATE 1

In comparing these figures illustrating the characteristics of endoxerosis and Alternaria rot of lemons, note especially the initial points of attack, the color differences, and the conditions of the affected tissues.

Fig. 1. A green, but practically mature, lemon showing an early stage of endoxerosis. Note deposits of gum near stylar end of lemon and in the peel at one side, and the more or less typical precoloring of the surface of the peel at the stylar end.

Figs. 2 and 3. Two different types of development in the later stages of endoxerosis.

Fig. 4. A comparatively early stage in the development of Alternaria rot. Note area of initial infection under the button, also the advancing area of infection down the center of the lemon.

Fig. 5. Intermediate stage in the development of Alternaria rot. Even at this stage it is not possible to detect the infection without cutting the lemon.

Fig. 6. A later stage of Alternaria rot. Following this stage the entire fruit becomes infected and becomes a dark-brown to almost black, soft mass of decaying tissues.

PLATE 1

PLATE 2

To visualize the region of the button or fruit from which figures 1 to 12, plates 2 and 3, were taken, reference should be made to text figure 1.

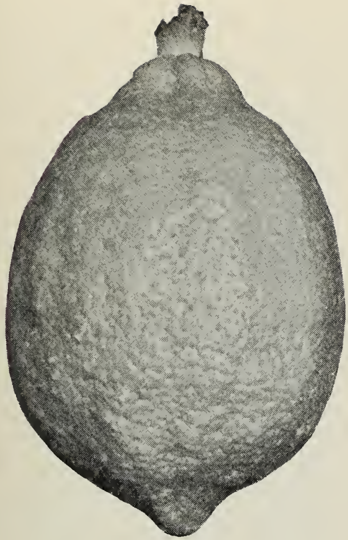
Figs. A and B. Mats of Alternaria mycelium growing from the buttons of lemons that had been stored where the temperature and humidity were too high.

Fig. 1. From the button of a silver lemon just after picking. Resistant spores and hyphae, below which cork cells have formed.

Fig. 2. From the button of a green lemon just after picking. Masses of resistant spores adhering to the surface of the button. The tissues below apparently unaffected.

Fig. 3. From the button of a silver lemon just after picking. Masses of resistant spores above the infected tissue which is penetrated by hyphae. Note resistant layer of cork cells below infected tissues.

Fig. 4. Portion of a crevice in a button from a green lemon that had been in storage ten days. During this period the temperature ranged from 75° to 85° F and the air was almost saturated with moisture. Note that there are hyphae in upper half of figure but none below the resistant corky layer in the lower half of figure.



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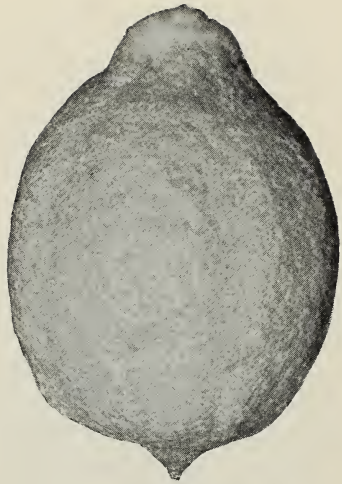

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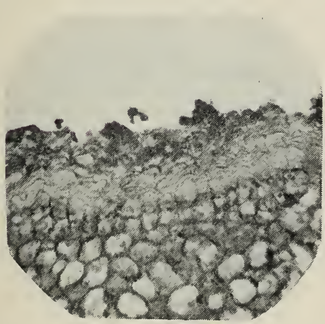

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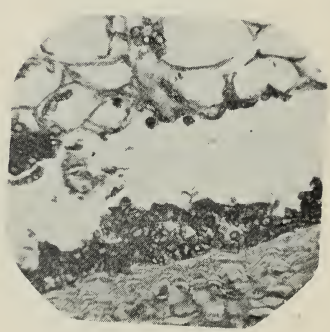

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PLATE 3

Fig. 5. From the button of a yellow lemon just after picking. Clumps of resistant hyphae formed on the surface of the infected tissues below.

Fig. 6. Source, same as for figure 5. Resistant hyphae in the infected tissues. Note again corky resistant layer below.

Fig. 7. Source, same as for figure 5. Portion of a crevice containing spores and hyphae; their advance at least retarded by the corky resistant layer.

Fig. 8. From a green lemon sweated ten days and then held in storage ten days longer. Enlarged ends of hyphae in one of the microscopical depressions or crevices which appear in the surface of the lemon under the button.

Fig. 9. From button of a green lemon just picked; button removed, placed in semesan sterilizing solution in 90 per cent vacuum for one hour, then plated on glucose-potato agar for eight days. Note vigorous growth of fungus in the tissues under these conditions.

Fig. 10. Source, same as for figure 8. Hyphae showing in a tangential section of the wood tissue of a button from a green lemon.

Fig. 11. Source, same as for figure 8. Hyphae in parenchyma tissue of lemon; about one-quarter inch below button.

Fig. 12. Source, same as for figure 9. Hyphae in cortex parenchyma of the lemon button.


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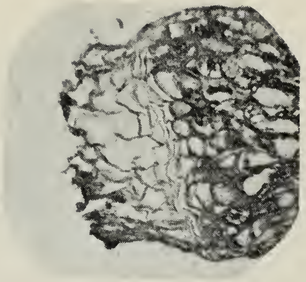
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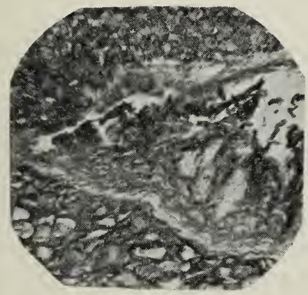
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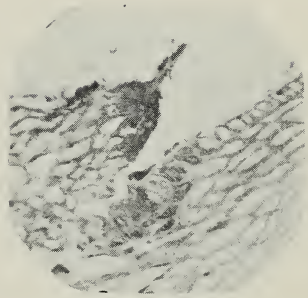
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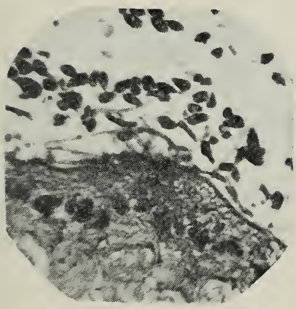
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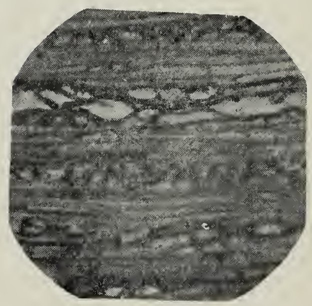
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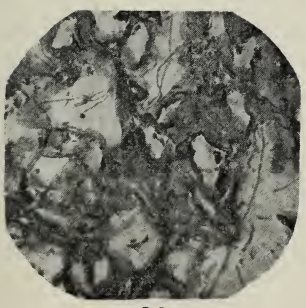
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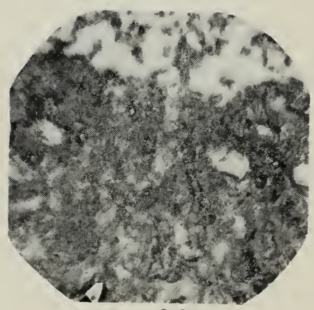
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